

Gallic Acid Isolated from Water Extracts of Litter from *Acer platanoides*

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It has been reported (Melin and Wikén 1946) that cold water extracts of dried and ground autumn leaves of *Acer platanoides* L. show antibacterial activity when tested against *Staphylococcus aureus*. The present communication describes the investigation of litter extracts of *Acer platanoides*, the isolation of the antibacterial substance and its identification as gallic acid.

There was a great difference in the activity of the water extract prepared by the above authors and those obtained in this investigation. Melin and Wikén report inhibition zones of 21—23 mm, whereas the present author did only obtain zones of 10—12 mm. Experiments were consequently initiated to find out the reason for this discrepancy. As it could be possible, that the higher activity depended on a different concentration of some antibiotic agent other than gallic acid, a careful investigation of the litter extracts was carried out. However, it was proved, that the activity was due only to gallic acid or secondary products derived from it. Other possible explanations of the difference in activity could be, that a hypothetic inhibitor is present only in the litter of certain years or special localities, or that it does only appear during a very short time in the autumn. The investigation was extended along these lines. Litter were collected over a period of several years in different parts of Uppsala as well as from certain places about 10 kilometers outside Uppsala, cold water extracts thereof were prepared and tested. However, none of them had an inhibition zone greater than 12 mm. The same negative result was given by the investigation of litter collected at intervals of a few days in the autumn.

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Table 1. *Effect of pH on the activity of gallic acid.*

pH	Inhibition zones in mm	
	Gallic acid	Standard solution
2.92	9.6	25.4
3.99	8.5	24.4
5.01	0	24.0
6.20	15.6	24.7
7.00	19.7	24.4

The assay values are average of 6 parallels.

During the investigation, it was discovered that the activity of gallic acid increased considerably when its water solution was neutralised with sodium hydroxide. An irreversible change occurs and the colourless aqueous solution turns deep green. The change in the activity with pH is shown in table 1. One per cent solution of gallic acid was used, and, as a standard, a penicillin solution containing 0.5 I.U. per ml.

It has long been known, that when gallic acid is treated with alkali in the presence of air, it is oxidised (cf. Schewket 1913). If the treatment is performed under special conditions, galloflavin $C_{12}H_6O_8$ is formed (Bohn and Graebe 1887, Haworth and McLachlan 1952). The structure of this compound has not yet been fully elucidated, but it is also sensitive to oxidation in alkaline media. These are the only information the author has been able to find concerning the oxidation products formed by gallic acid when treated with alkali, and the matter was not further investigated.

From table 1 it is evident, that the activity of gallic acid is rather low but that it is increased when gallic acid is mixed with some of its oxidation products. This might possibly be due to an intermediate substance, perhaps galloflavin, as it has also been found that there are some differences in activity, if aqueous solutions of gallic acid are neutralised to pH 7, or if gallic acid is first dissolved in sodium hydroxide and then neutralised to pH 7; the first mentioned solutions being always more active.

A quite satisfactory explanation of the observed difference in activity of the litter extracts has not been found; however, it might be possible that the higher activity is due to a pH effect. The extracts prepared by the author had a pH of about 4.0—4.5, at which pH, according to table 1, the activity of gallic acid is close to its minimum value. The increase of activity reported by Melin and Wikén to occur when extracts were autoclaved, may perhaps be due to the formation of more oxidation products. Other chemical changes can also influence the activity, as gallic acid is destroyed when autoclaved or boiled in aqueous solutions, pyrogallol being formed (Widmer 1929).

Gallic acid commonly occurs in higher plants, usually in form of tannin glycosides, and as such it has been isolated from other species of *Acer*, viz. *Acer aizuenes* (Tsujimoto 1951) and *Acer ginnala* (Perkin and Uyeda 1922). As in the case of most phenol compounds, the antibacterial activity of gallic acid has long been known. In recent years ethyl gallate, isolated from the leaves of *Haematoxylon campechianum* (Little *et al.* 1953), has been found to be active against *Mycobacterium tuberculosis*. Also in this case, oxidation products seem to play an important role, for ethyl gallate has optimal activity at pH 6.4.

Experimental

The leaves were collected from the ground in the autumn when their colour had turned yellow-brown; they were air-dried, ground and thoroughly mixed with distilled water in the proportion 1 : 5. The mixture was placed in a refrigerator for about 20 hours and was then filtered. The filtrate which contains about 2 per cent of solid material and had a pH of about 4.0—4.5 was mixed with 2 per cent by weight of Norite, which adsorbs all the active material, and left over night. The next day it was centrifuged, and the Norite was washed with a small quantity of water. The active material was eluted from the Norite with 80 per cent aqueous acetone, and from the eluate the acetone and some of the water were evaporated. The resulting solution was acidified with hydrochloric acid, saturated with sodium chloride and shaken with ethyl acetate which removed the active material. By concentrating the ethyl acetate to a small volume and adding petroleum ether, the active material was precipitated as a yellow powder. This was further purified by dissolving in ethyl acetate, treating with Norite and reprecipitating with petroleum ether. The precipitate was recrystallised with hot water and separated as fine colourless needles which, when heated in a capillary tube, decomposed without melting at about 220°. The elementary analysis gave C 49.74 and H 3.67 per cent and the electrometric titration an equivalent weight of 170.9. Gallic acid, whose formula ($C_7H_6O_5$) requires C 49.42 and H 3.56, has an equivalent weight of 170.1. The ethyl ester of the isolated acid was also prepared; it melted at 154° and when mixed with authentic ethyl gallate, there was no depression in the melting point.

As there was a slight possibility, that some other antibacterial substance could be present in the dried leaves, aqueous litter extract as well as alcohol and acetone extracts thereof were thoroughly investigated by means of chromatographic adsorption, distillation with steam, extractions with different organic solvents and other techniques of separation. It was, however, found, that all active solutions contained gallic acid, and that all activity could be traced back to that acid.

In order to find out whether the litter extract could possibly have optimal activity only during a very short time in the autumn, leaves were collected at intervals of a few days over a period of three weeks partly from the ground and partly from a selected tree. Some of the leaves were air-dried, ground and prepared as above, others were, without drying, treated with distilled water in the proportion 1 : 5 in a turmix desintegrator; the mixture was then filtered and tested. The results are presented in table 2.

Table 2. Inhibition zones in mm. on different days.

Material		Date October					
		1	4	8	10	15	24
leaves from the ground	I	+ ¹	+	9.2	9.3	9.7	7.3
	II	+	+	+	+	10.5	
leaves from the tree	I	+	7.7	+	8	10	
	II	+	+	+	+	7.7	

I=air-dried leaves.

II=leaves treated in the desintegrator.

¹ inhibition only within the test cylinder (diameter 7 mm).

Summary

Gallic acid has been isolated from litter extracts of *Acer platanoides*. It has low activity when tested against *Staphylococcus aureus*, but the activity is increased when gallic acid is neutralised with alkali. The optimal activity is found at pH 7 in aqueous solutions and may depend on the presence of some oxidation products formed when gallic acid is treated with alkali.

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